

AMENDMENTS TO THE CLAIMS

1. (currently amended): An isolated chimeric protein having the enzymatic activity of a nucleotidase, which chimeric protein comprises, from N-terminus to C-terminus:

a) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

b) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3' (2'), 5'-bisphosphonate activity of SEQ ID NO:2; and

c) a third peptidyl fragment comprising the amino acid sequence of SEQ ID NO:3.

2-11. (canceled)

12. (previously presented): The isolated chimeric protein of claim 1, wherein the first and second peptidyl fragments are linked via a cleavable linkage.

13-20. (canceled)

21. (previously presented): The isolated chimeric protein of claim 1, which further comprises, at its C-terminus a fourth peptidyl fragment comprising a peptide tag.

22. (previously presented): The isolated chimeric protein of claim 21, wherein the peptide tag is selected from the group consisting of FLAG, HA HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, ϵ , B, gE, and Tyl tag.

23. (previously presented): An isolated chimeric protein which comprises the amino acid sequence set forth in SEQ ID NO:4 (mgsggdddlalALERELLVATQAVRKASLLTKRIQSEVI SHKDSTTITKNDNSPVTG DYAAQTIIINAISNFPDDKVVGEESSGLSDAFVSGILNEIKAN DEVYNKNYKKDDFLFTNDQFPLKSLEDVRQIIDFGNYEGGRKGRFWCLDPIDGTGKFLRGE

QFAVCLALIVDGVVQLGCIGCPNLVLSSYGAQDLKGHESFGYIFRAVRGLGAFYSPSSDAES
 WTKIHVRHLKDTKDMITLEGVEKGHSSHDEQTAIKNKNLSKSLHLSQAKYCLLALGLAD
 VYLRLPIKLSYQEKIWDHAAGNVIVHEAGGIHTDAMEDVPLDFGNGRTLATKGVIASSGPR
 ELHDLVVSTSCDVIQSRNAkegleglpipnpllrghhhhhh).

24-30. (canceled)

31. (currently amended): A method for assaying for sodium ions in a sample, which method comprises:

a) contacting the sample with a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of in SEQ ID NO:3;

wherein the chimeric protein comprises a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase, wherein the nucleotidase consumes adenosine 3',5'-bisphosphate (PAP) and forms AMP and P_i; and

b) assessing the consumption of PAP or the formation of AMP or P_i in step a) to determine the presence or amount of sodium ions in the sample.

32. (original): The method of claim 31, wherein the sample is a biological sample.

33. (original): The method of claim 32, wherein the biological sample is a blood sample.

34. (original): The method of claim 33, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

35-36. (canceled)

37. (original): The method of claim 31, wherein the amount of AMP formed is inversely related to the amount of sodium ions in the sample.

38. (original): The method of claim 31, which is used in prognosis or diagnosis of a disease or disorder.

39. (currently amended): A method for assaying for sodium ions in a sample, which method comprises:

a) contacting the sample with a first composition comprising adenosine 3',5'-bisphosphate (PAP);

b) contacting the sample with a second composition comprising a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of SEQ ID NO:3;

wherein the chimeric protein comprises a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and

c) assaying the production of AMP to determine the presence or amount of sodium ions in the sample.

40. (original): The method of claim 39, wherein the sample is a biological sample.

41. (original): The method of claim 40, wherein the biological sample is a blood sample.

42. (original): The method of claim 41, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

43. (canceled)

44. (original): The method of claim 39, wherein the first composition further comprises 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, and the second composition further comprises adenosine deaminase, 5'-nucleotidase, and $MgCl_2$.

45. (currently amended): A kit for assaying for sodium ions in a sample, which kit comprises:

a) a first composition comprising a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of in SEQ ID NO:3;

wherein the chimeric protein comprises a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase that consumes adenosine 3',5'-bisphosphate and forms AMP and P_i ; and

b) means for assessing the product formed or the substrate consumed by the nucleotidase to determine the presence or amount of the sodium ions in the sample.

46. (original): The kit of claim 45, wherein the first composition further comprises adenosine deaminase, 5'-nucleotidase and $MgCl_2$.

47. (previously presented): The kit of claim 45, further comprising a second composition comprising 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, wherein the reaction of 4-AA and EHSPT in the presence of peroxidase is the means for assessing the product formed.

48. (original): The kit of claim 45, which further comprises a low sodium serum standard and a high sodium serum standard.

49. (canceled)

50. (currently amended): A method for assaying for lithium ions in a sample, which method comprises:

a) contacting the sample with a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of SEQ ID NO:3;

wherein the chimeric protein comprises a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase, wherein the nucleotidase consumes adenosine 3',5'-bisphosphate (PAP) and forms AMP and P_i; and

b) assessing the amount of PAP consumed or AMP or P_i formed in step (a) to determine the presence or absence of lithium ions in the sample.

51. (original): The method of claim 50 further comprising first contacting the sample with a sodium blocking agent.

52. (original): The method of claim 51, wherein the sodium blocking agent is 4, 7, 13, 16, 21-pentaoxa-1,10-diazabicyclo[8.8.5]-tricosane.

53. (original): The method of claim 51, wherein the sample is a biological sample.

54. (original): The method of claim 53, wherein the biological sample is a blood sample.

55. (original): The method of claim 54, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

56-57. (canceled)

58. (original): The method of claim 51, wherein the amount of AMP formed is inversely correlated to the amount of lithium ions in the sample.

59. (original): The method of claim 51, which is used in prognosis or diagnosis of a disease or disorder.

60. (currently amended): A method for assaying for lithium ions in a sample, which method comprises:

a) contacting the sample with a first composition comprising adenosine 3',5'-bisphosphate (PAP);

b) contacting the sample with a second composition comprising a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of SEQ ID NO:3;

wherein the chimeric protein comprises a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and

c) assessing the production of a detectable product to determine the presence or absence of lithium ions in the sample.

61. (original): The method of claim 60 further comprising first contacting the sample with a sodium blocking agent.

62. (original): The method of claim 61, wherein the sodium blocking agent is 4, 7, 13, 16, 21-pentaoxa-1,10-diazabicyclo[8.8.5]-tricosane.

63. (original): The method of claim 60, wherein the sample is a biological sample.

64. (original): The method of claim 63, wherein the biological sample is a blood sample.

65. (original): The method of claim 64, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

66. (canceled)

67. (original): The method of claim 60, wherein the first composition further comprises 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, and the second composition further comprises adenosine deaminase, 5'-nucleotidase, and $MgCl_2$.

68. (currently amended): A kit for assaying for lithium ion in a sample, which kit comprises:

a) a first composition comprising a chimeric protein comprising, from N-terminus to C-terminus:

(i) a first peptidyl fragment comprising a bacterial leader sequence comprising the amino acid sequence of SEQ ID NO:1;

(ii) a second peptidyl fragment comprising the amino acid sequence of SEQ ID NO:2 or comprising the amino acid sequence of SEQ ID NO:2 having a single no more than one conservative amino acid substitution, wherein the substituted peptidyl fragment retains at least 90% of the 3'(2'),5'-bisphosphonate activity of SEQ ID NO:2; and

(iii) a third peptidyl fragment comprising the amino acid sequence of SEQ ID NO:3;

wherein the chimeric protein comprises a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and

b) a means for assessing the adenosine 3',5'-bisphosphate consumed or the AMP or Pi formed by the 3'(2'),5'-bisphosphate nucleotidase to determine the presence or amount of said lithium ions in the sample.

69. (previously presented): The kit of claim 68 further comprising a sodium blocking agent.

70. (original): The kit of claim 68, wherein the first composition further comprises adenosine deaminase, 5'-nucleotidase and $MgCl_2$.

71. (previously presented): The kit of claim 68, further comprising a second composition comprising 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfoethyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, wherein the reaction of 4-AA and EHSPT in the presence of peroxidase is the means for assessing the product formed.

72. (original): The kit of claim 68, which further comprises a low lithium serum standard, a medium lithium sodium standard, and a high lithium serum standard.